

AMENDMENTS TO THE SPECIFICATION

Page 1, line 2, below "TITLE OF INVENTION", please replace the current title with the following title:

--A Plexin family-like polypeptide, DNA encoding the same and uses thereof--

Pages 3-4, please amend numbered paragraph [0005] as follows:

[0005]

Human KIAA0620 gene is located on the chromosome 3q21.3, and several SNPs of the human KIAA0620 gene are reported

(~~http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide&cmd=Display&dopt=nucleotide_snp&from_uid=3327053~~). As majority of human diseases are not caused by mere deletion of a specific gene, but by partial alteration in functions or activities of a protein by amino acid substitution, it is suspected that human KIAA0620 gene may be involved in healing of wound, healing of fracture, vascular occlusion and collateral vessel formation, periodic formation of vascular network in tunica mucosa uteri (transient or at the time of luteinization); various processes in which angiogenesis is undesirably involved such as proliferation of cancer cells, chronic articular rheumatism, diabetic retinopathy, endometriosis, obesity; and various processes in which angiogenesis is desirably involved such as heart attack, neurodegenerative diseases, circulatory deficits in legs, arteriosclerosis obliterans, and psoriasis vulgaris. Although it was reported that mRNA of the gene relating to human KIAA0620 gene is expressed in vascular endothelial cells or central nervous system (CNS) in a mouse during development process, researches using not human but experimental animals are essential to elucidate human KIAA0602 gene and its relationship. However, the gene that corresponds to human KIAA0620 gene has not been obtained yet from rodents, particularly from a mouse which is an important model animal in researches of human pathology, thus a study that uses the gene could not have been conducted so far.

Brief Description of Drawings Section:

Page 13, beginning on the last line, please amend numbered paragraph [0021] as follows:

Brief description of the drawings

[0021]

Figure 1 illustrates Semaphorin/CD100 antigen domain, three Plexin/Semaphorin/integrin domains, three cell surface receptor IPT/TIG domains, and a transmembrane (TM) segment identified in novel Plexin polypeptide of the invention (aa: amino acid sequence **(SEQ ID NO. 18)**) wherein the figure represents the number of amino acids. Box: domains and transmembrane (TM) segment identified by different search methods. Searches used are, from top to bottom, HMMPfam search, HMMSmart search, and Sosui search). From these results, novel Plexin polypeptide **(SEQ ID NO. 18)** was identified by HMMPfam search, HMMSmart search, and Sosui search.

Figure 2A illustrates expression sites of mpf00920 (mouse KIAA0620) gene in various development stages of a mouse 10.5d. p. c., detected by the whole mount in situ method. Illustrated is an image detected by a negative control using a sense probe (expression sites are represented by deep stained region along the vessel).

Figure 2B illustrates expression sites of mpf00920 (mouse KIAA0620) gene in various development stages of a mouse 10.5d. p. c., detected by the whole mount in situ method. Illustrated is the expression intensity of mpf00920 gene detected by using an antisense probe (expression sites are represented by deep stained region along the vessel).

Figure 2C illustrates expression sites of mpf00920 (mouse KIAA0620) gene in various development stages of a mouse 8 d. p. c., detected by the whole mount in situ method (expression sites are represented by deep stained region along the vessel).

Figure 2D illustrates expression sites of mpf00920 (mouse KIAA0620) gene in various development stages of a mouse 9.5d. p. c., detected by the whole mount in situ method (deep stained region can be identified along the vessel).

Figure 3A₁ illustrates expression sites of mpf00920 (mouse KIAA0620) gene in the sagittal direction of fetus frozen section 14.5 days after fertilization detected by the in situ method. The

top view illustrates an image detected by the negative control using a sense probe[[],]. Figure 3A2, ~~while the bottom view~~ illustrates expression intensity of mpf00920 gene detected by using an antisense probe (expression sites are represented by deep stained region).

Figure 3B is a highly enlarged image showing expression intensity of mpf00920 gene in the brain detected by using an antisense probe (expression sites are represented by deep stained region).

Figure 4A illustrates expression sites of mpf00920 (mouse KIAA0620) gene represented by mRNA level in a paraffin section of a stomach of an adult mouse detected by the in situ method. The stratified-squamous-epithelia site is deeply stained.

Figure 4B illustrates expression sites of mpf00920 (mouse KIAA0620) gene represented by mRNA level in a paraffin section of a cerebrum of an adult mouse detected by the in situ method. The cerebral neuroglia cells are deeply stained.

Figure 4C illustrates expression sites of mpf00920 (mouse KIAA0620) gene represented by mRNA level in a paraffin section of a cerebellum of an adult mouse detected by the in situ method. The cerebellar Purkinje cell layer is deeply stained.

Figure 5 is a photograph showing electrophoresis which compares expression frequency of mouse KIAA0620 gene in mouse fetus development process represented by mRNA level detected by RT-PCR method. The top view illustrates expression intensity of Flk1 gene used as a control, and the bottom view illustrates the expression intensity of mouse KIAA0620 gene. The expression intensity is represented by ethidium bromid staining pattern after fractioning by agarose electrophoresis.

Figure 6 is a photograph of a membrane filter which shows the result of detection of lacZ-V5 fusion protein (lacZ-V5 proteins) and mouse KIAA0620-V5 fusion protein (mKIAA0620-cds-V5 protein) which are generated in host HEK293 cells detected by Western blot with use of a rabbit polyclonal antibody. In the figure, M is a high range marker, 1 is HEK293 total proteins, 2 is lacZ-V5 protein, 3 is mKIAA0620-cds-V5 protein (these 1 - 4 lanes were stained with anti-V5-HRP antibody); 4 is HEK293 total proteins, 5 is lacZ-V5 protein, 6 is mKIAA0620-cds-V5 protein, 7 is HEK293 total protein, 8 is lacZ-V5 protein, 9 is mKIAA0620-cds-V5 protein (these

5 - 8 lanes were stained with anti-mKIAA0620 antibody). The numerical values in the figures indicate dilution factors.

Figure 7 is a microscopy photograph that shows the result of detection of mouse KIAA0620 gene expression in the retinal blood vessels of a neonatal mouse detected by the in situ hybridization method (left: two days after the birth, central: four days after the birth, right: seven days after the birth. Deeply stained sites can be identified along the newly generated vessels).

Figures 8A-B are is-a photographs that shows show the result of Western blot analysis of the whole protein fraction and the hydrophobic fraction extracted from the whole protein fraction. The protein was generated by using HEK 293 cells as a host, and expressing the mouse KIAA0620 full length - V5 fusion protein and a mouse extramembrane segment containing transmembrane segment (TM), that is, mouse KIAA0620 extramembrane segment (TM) - V5 fusion protein. In the figure, 1 represents the analysis result of the whole protein fraction prepared from the control cells, 2 represents that of the hydrophobic protein fraction prepared from the control cells, 3 represents that of the whole protein fractions prepared from the cells which express mouse KIAA0620 full length - V5 fusion protein , 4 represents that of the hydrophobic protein fraction from the cells which express mouse KIAA0620 full length - V5 fusion protein, 5 represents that of the whole protein fraction prepared from the cells which express mouse KIAA0620 extramembrane segment (TM) - V5 fusion protein, and 6 represents that of the hydrophobic protein fraction prepared from the cells which express mouse KIAA0620 extramembrane segment (TM) - V5 fusion protein. The photograph on the left, Figure 8A is an image obtained by color development, that on the right, Figure 8B is an image obtained by chemiluminescence, and the ladder and numerical values in the center indicate the size marker and molecular weight (kDa) represented by the marker.

Figures 9A-D are is-a microscopy photographs which shows show the result of the experiment to inhibit development of retinal vessels in a neonatal mouse using mouse KIAA0620 extramembrane segment - IgG1Fc fusion protein five hours after the injection on the fifth day after the birth). Five hours after injection into the eyeball, excessive formation of filopodia was observed in the retinal blood vessels on the way of development, especially in the

endothelial cells at the leading end of developing vessels (~~lower left (Figure 9C), lower right of the figure (Figure 9D)~~ : mKIAA0620 extramembrane segment - Fc). On the other hand, no change was observed in the eyeball of a mouse to which only IgG1Fc protein was injected (~~upper left (Figure 9A), (Figure 9B) upper right in the figure:~~ Fc).

Figures 10A-B ~~are~~ is-a microscopy photographs which shows the result of an experiment to inhibit development of retinal vessels in a neonatal mouse using mouse KIAA0620 extramembrane segment – IgG1Fc fusion protein (three days after the injection which was performed one day after the birth). Three days after the injection into the eyeball, the construction of vascular network of retina was significantly disturbed (Figure 10 B ~~right side of the figure,~~ mKIAA0620 extramembrane segment - Fc). On the other hand, no change was observed in the eyeball of a mouse to which only IgG1Fc protein was injected (Figure 10A ~~upper left in the figure:~~ Fc).

Pages 24-25, please amend numbered paragraph [0036] as follows:

[0036]

As the expression vector to be used for gene insertion to animal cells for the purpose of producing recombinant animal cells, the expression vectors as described above and vectors known in the art can be used that have Bluescript SK (+/-) vector as a template and linking Tie2 promoter (a promoter specific to vascular endothelial cells) in order to ensure expression specific in vascular endothelial cells. Endothelial cell specific expression can be achieved by using these expression vectors and promoters. Alternatively, a cloning system known as ~~Gateway~~ (~~trademark~~) GATEWAY™ cloning technology (Invitrogen: Cat. No.11821-014) can also be used.

Pages 61-62, please amend numbered paragraph [0111] as follows:

[0111]

(6) Producing transgenic cells

With use of mpf00921 gene sequence represented by SEQ ID NO: 2 (non full-length cDNA lacking 5'-terminal sequence: 6,178 bps), and PCR product comprising 5'-terminal DNA fragment (SEQ ID NO: 10) obtained in above (5), a full-length cDNA (SEQ ID NO: 16) corresponding to ORF was obtained according to the following procedure. First, mpf00921 gene sequence represented by SEQ ID NO: 2 (non full-length cDNA of mouse KIAA620) was inserted into a multi-cloning site of pBluescript SK (+) to prepare a recombinant plasmid. Particularly, with use of Bam HI site in the sequence of multi-cloning site of pBluescript SK (+), 5'-terminal DNA fragment which had been cut by same restricted enzyme was designed and ligated for alignment to the frame. Then, E. coli (DH 5 α strain) was transformed to generate a plasmid DNA containing full-length DNA fragment of interest. The plasmid containing full-length ORF cDNA (5,992 bps. A sequence that encodes a polypeptide of 1,996 amino acid, plus stop codon) was transformed into E. coli, and purified and collected. A plurality of expression construct was prepared using this plasmid as a template. For preparing the expression construct, Gateway GATEWAYTM cloning technology INVITROGENTM (~~Invitrogen~~: Cat. No. 11821-014) system was used, and an entry clone comprising following target PCR product incorporated into pENTR/D-TOPO Vector INVITROGENTM (~~Invitrogen~~). PCR product to be used for native protein was prepared by designing and synthesizing following primer as full-length cDNA (from initiation codon to stop codon), and conducting PCR reaction. Particularly, with use of CACCATgggctgtgggcgtgtct (SEQ ID NO: 12, 24 bps. The sixth to 25th base (20 bps) in the sequence represented by SEQ ID NO: 16) as a forward primer, and Reverse primer 1 (SEQ ID NO: 13, TCAGGCCTCGCTGTAACACTCATAGA, 26 bps. A sequence complementary to 26 bps sequence from the 5,218th to the 5,243rd base in the sequence represented by SEQ ID NO: 2, including a stop codon) as a reverse primer, entry clone was prepared. Tagged PCR product to be used for tagged fusion protein was prepared by designing, synthesizing and using the following primer as a tagged full-length cDNA (from initiation codon to stop codon), by PCR reaction. Particularly, with use of above Forward primer (SEQ ID NO: 12,

CACCA~~tg~~gggctgtgggcgtgtgtct, 24 bps. The 6th to 25th base (20 bps) in the sequence represented by SEQ ID NO: 16) as a forward primer, and Reverse primer 2 (SEQ ID NO: 14, GGCTCGCTGTAACACTCATAGA, 23 bps. A sequence complementary to 23 bps sequence from the 5,218th to the 5,240th base in the sequence represented by SEQ ID NO: 2, not including a stop codon) as a reverse primer, entry clone was prepared.

Pages 64-65, please amend numbered paragraph [0115] as follows:

[0115]

(7-2)

Detection of mouse KIAA0620 protein according to Western blotting in various transgenic cells using purified rabbit polyclonal antibody

With use of the purified rabbit polyclonal antibody obtained as above, mouse KIAA0620 protein was detected in various transgenic cells according to the Western blotting. The protein was collected from the transgenic cells according to the following procedures. In order to collect protein from the transgenic cells, HEK293T cells to which mouse KIAA0620 gene compulsive expression vector was introduced were washed on a culture dish with PBS(-) solution, and solubilized by repeated pipetting with Lysis Buffer (TNE buffer). The solubilized solution was introduced into 28G injection needle. After centrifugation (700g, 10 min), supernatant was collected as a sample solution which contained protein from mouse KIAA0620 gene transgenic cells. Other sample solution was prepared in similar manner using HEK293T cells into which expression vector which expresses mouse KIAA0620 - V5 fusion protein was introduced. Sample solutions were fragmented by polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was conducted as follows. About 20 micrograms of different proteins derived from different transgenic cells were applied to each lane, and polyacrylamide gel electrophoresis was performed to fraction the sample. Then fractioned proteins were plotted on membrane. On the plotted membrane, immunostaining reaction was performed using 500-fold or 1000-fold diluted antibody. Polyacrylamide gel electrophoresis was conducted using 4 - 12% Tris-Glycine Gel (#NO. EC6035Box) supplied from ~~Invitrogen~~ INVITROGENTM. Protein was transferred

using the semi-dry transfer cell supplied from Bio Rad Co., Ltd, and plotted on the membrane. As second antibody, 5000-fold diluted Goat Anti-Rabbit IgG, HRP-conjugate antibody BIOSOURCETM (#No. ALI0404, ~~Biosource~~TM) was used, and for detection, ECL Western blotting Detection Reagents BIOSOURCETM (#No. RPN2133, ~~Biosource~~TM) was used. In order to determine molecular weight, a pre-stained protein marker supplied from Nacalai Tesque (High Range, SDS-PAGE (#No. 26039-75) was electrophoresed together with each sample.

Pages 81-82, please amend numbered paragraph [0148] as follows:

[0148]

(13)

Analysis of polypeptide function of the invention in angiogenesis in the retina of a neonatal mouse using mouse KIAA620 protein extramembrane region - IgG1Fc fusion protein

The extramembrane region polypeptide having 1,337 amino acids (identical to the sequence from the 2nd to the 1,338 amino acid in the sequence represented by SEQ ID NO: 15) was fused to human IgG1Fc fragment region using gene recombination method, and a plasmid vector which expresses the product of this gene under the control of CAG promoter. The plasmid vector was introduced to 293T cells using Trans IT-LT1 Transfection Reagents supplied by Mirus Bio Corporation. The resultant cells were cultured for 3 days in a serum free medium INVITROGENTM (GibcoCD293, ~~Invitrogen~~). The culture was applied to Protein G column (~~Hi Trap~~ (TM) HI TRAPTM Protein G HP, Amersham Biosciences) to purify mouse KIAA0620 extramembrane region - IgG1Fc fusion protein from the supernatant of the culture.